

U.S. Ser. No. 09/673,779 -7-
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WHAT IS CLAIMED IS:

1. (Amended) A method for identifying the presence of a bacterium in a sample comprising
 - a) testing said sample by Gram-staining and
 - b) testing said sample with a probe according to an *in situ* hybridisation protocol selected on the basis of the outcome of said Gram-staining and identifying the presence of the bacterium in the sample.
2. A method according to claim 1 wherein said sample is a clinical sample.
3. (Amended) A method according to claim 2 wherein said sample is mammalian blood.
4. (Twice Amended) A method according to claim 1 when said Gram-staining indicates the presence of a Gram-negative bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.
5. (Amended) A method according to claim 4 wherein said character is of the rod type, further comprising hybridising said sample with at least one probe selected from a group consisting of probes capable of hybridising with nucleic acid found *Escherichia coli*, in *Klebsiella pneumoniae*, in *Klebsiella oxytoca*, in *Serratia marcescens*, in *Enterobacter aerogenes*, in *Enterobacter cloacae*, in *Proteus vulgaris*, in *Proteus mirabilis*, in *Salmonella typhi*, in *Pseudomonas aeruginosa*.

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7. (Twice Amended) A method according to claim 6 wherein said probe is having no more than five mismatches with a probe selected of a group consisting of probes having a sequence GCCTGCCAGTTTCGAATG (SEQ ID NO:1) or GTAGCCCTACTCGTAAGG (SEQ ID NO:2) or GAGCAAAGGTATTAACCTTACTCCC (SEQ ID NO:3) or GTTAGCCGTCCCTTCTGG (SEQ ID NO:4).
8. A method according to claim 4 wherein said character is of the coccus type, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme.
9. (Twice Amended) A method according to claim 1, when said Gram-staining indicates the presence of a Gram-positive bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.
10. A method according to claim 9 wherein said character is of the rod type, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme and/or Proteinase K.
11. (Amended) A method according to claim 9 wherein said character is of the coccus type, further comprising determining a chain-like or clump-like character of said bacteria.
12. A method according to claim 11 wherein said character is chain-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme.
13. (Amended) A method according to claim 12 further comprising hybridising said sample with at least one probe selected from a group consisting of probes capable of

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14. A method according to claim 13 wherein said nucleic acid is ribosomal RNA.
15. (Twice Amended) A method according to claim 14 wherein said probe is having no more than five mismatches with a probe selected of a group composed of probes having a sequence TTATCCCCCTCTGATGGG (SEQ ID NO:5) or AGAGAAGCAAGCTTCTCGTCCG (SEQ ID NO:10) or GCCACTCCTCTTTCCGG (SEQ ID NO:7).
16. A method according to claim 11 wherein said character is clump-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysostaphin and/or Proteinase K.
17. (Amended) A method according to claim 16 further comprising hybridising said sample with at least one probe selected from a group consisting of probes capable of hybridising with nucleic acid found in *Staphylococcus aureus*, in *Staphylococcus haemolyticus*, in *Staphylococcus saprophyticus*.
18. A method according to claim 17 wherein said nucleic acid is ribosomal RNA.
19. (Twice Amended) A method according to claim 18 wherein said probe is having no more than five mismatches with a probe selected of a group consisting of probes having a sequence GCTAATGCAGCGCGGATCC (SEQ ID NO:8) or CCGAAGGGGAAGGCTCTA (SEQ ID NO:9) or AGAGAAGCAAGCTTCTCGTCCGTT (SEQ ID NO:10).

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20. (Twice Amended) A method according to claim 4 further comprising hybridising said sample with at least one positive control probe and/or with at least one negative control probe.

21. (Amended) A method according to claim 20 wherein said positive control probe comprising no more than five mismatches with a probe with the sequence GCTGCCTCCCGTAGGAGT (SEQ ID NO:11) and/or wherein said negative control probe comprises no more than five mismatches with a probe with the sequence ACTCCTACGGGAGGCAGC (SEQ ID NO:12).

22. (Twice Amended) A method according to claim 1 further comprising a one-step procedure of binding bacteria present in said sample to a microscopic slide and simultaneously fixing intracellular structures.

23. (Twice Amended) A method according to claim 1 wherein said probe is selected for its properties of reactivity with a selected one or more of bacterial genera and/or species including a consideration of the susceptibility to antibiotic treatment of said probe.

Amend ① = Preliminary Amendment filed 10/19/00. Claims 4, 9, 20, 22, 23

Amend. ⑥ = Second Preliminary Amendment filed 3-19-01 (Claims 7, 15, 19, 21, 25)

Amend (C) = Amendment filed March 21, 2002 (Claims 13, 4, 5, 7, 9, 11, 13, 15, 17, 19, 20, 22, 23)

(Amended) A method for [determining] a bacterium [suspected of being present] in a sample comprising

a) testing said sample by Gram-staining and

b) testing said sample with a probe according to an *in situ* hybridisation protocol selected on the basis of a

3164 hybridisation process selected on the basis of the outcome of said Gram-staining[7] and identifying the presence of the bacterium

2. A method according to claim 1 wherein said sample is a clinical sample.

critical sample.
Amended

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3. A method according to claim 2 wherein said sample is mammalian blood, preferably being derived from a human.

4. A method according to claim 1, 2 or 3 [wherein] ^{when} said

Gram-staining indicates the presence of a Gram-negative bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.
(Arched)

5. A method according to claim 4 wherein said character is (D)

of the rod type, further comprising hybridising said sample with at least one probe selected from a group of probes capable of hybridising with nucleic acid found in *Escherichia coli*, in *Klebsiella pneumoniae*, in *Klebsiella oxytoca*, in *Serratia marcescens*, in *Enterobacter aerogenes*, in *Enterobacter cloacae*, in *Proteus vulgaris*, in *Proteus mirabilis*, in *Salmonella typhi*, in *Pseudomonas aeruginosa*.

6. A method according to claim 5 wherein said nucleic acid is ribosomal RNA.

7. A method according to claim 6 wherein said probe is having no more than five, [preferably no more than two] mismatches with a probe selected of a group [composed] of probes having a sequence $\text{GCCTGCCAGTTCTGAATG}$, or $\text{GTAGGCCCTACTCGTAAGG}$, or $\text{GAGCAAAGGTATTAACTTTACTCCG}$, or $\text{GTTAGCCGTCCCTTTCTGG}$. (SEQ ID NO: 1) (SEQ ID NO: 2) (SEQ ID NO: 3) (SEQ ID NO: 4)

9. A method according to claim 4 wherein said character is of the coccus type, further comprising subjecting said sample to treatment with a lysis buffer comprising

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bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.

10. A method according to claim 9 wherein said character is of the rod type, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme and/or pronase.

lysozyme and/or Proteinase K.

11.) A method according to claim 9 wherein said character is of the coccus type, further comprising determining a chain-like or clump-like character of said bacteria. (D) (C)

12. A method according to claim 11 wherein said character is chain-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme.

13. A method according to claim 12 further comprising hybridising said sample with at least one probe selected from a group ^{consisting} of probes capable of hybridising with nucleic acid found in *Enterococcus faecalis*, in *Streptococcus pneumoniae*, in *Streptococcus mitis*, in *Streptococcus viridans*, in *Streptococcus sanguis*, in *Enterococcus faecium*. (D) (C)

14. A method according to claim 13 wherein said nucleic acid is ribosomal RNA.

15. A method according to claim 14 wherein said probe is having no more than five, preferably no more than two mismatches with a probe selected of a group composed of probes having a sequence TTATCCCCCTCTGATGGG or AGAGAAAGCAAGCTTCTCGTCCG or GCCACTCCTCTTTTCCGG.

16. A method according to claim 11 wherein said character is clump-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme and/or Protease K.

17. A method according to claim 16 further comprising
hybridising said sample with at least one probe selected
from a group ^{consisting} of probes capable of hybridising with nucleic
acid found in *Staphylococcus aureus*, in *Staphylococcus*
haemolyticus, in *Staphylococcus saprophyticus*.

18. A method according to claim 17,

(Amended)

19. A method according to claim 18 wherein said probe is having no more than five, preferably no more than two

mismatches with a probe selected of a group [composed of] consisting of probes having a sequence GCTAAATGCAGGGCGGATCC or (SEQ ID No.8) CGGAAGGGCAAGGCTCTA, or AGAGAAGCAAGCTCTCGTCCTG (SEQ ID No.9) (Twice Amended)

20. A method according to [any of] claims 4 to 19 further comprising hybridising said sample with at least one positive control probe and/or with at least one negative control probe.

(Amended) 21. A method according to claim 20 wherein said positive control probe comprises no more than five mismatches with a probe with the sequence GCTGCCCTCCCGTAGGAGT, and/or wherein said negative control probe comprises no more than five mismatches with a probe with the sequence

ACTCCTACGGGAGGCAGC (SEQ ID No.12)

(Twice Amended) 22. A method according to [anyone of] claims 1 to 21 further comprising a one-step procedure [to bind] bacteria present in said sample to a microscopic slide and simultaneously fix^{ing} intracellular structures.

(Twice Amended) 23. A method according to [anyone of] claims 1 to 22 wherein said probe is selected for its reactivity with a selected one or [a group of] bacterial genera and/or species [having properties of] including a consideration of the congruent susceptibility to antibiotic treatment of said probe

24. A probe detecting or identifying a bacterium in a sample, preferably a clinical sample, said probe designed to hybridise specifically with nucleic acid in bacteria with congruent susceptibility or resistance to antibiotics.

(Amended) 25. A probe according to claim 24 wherein said probe is having no more than five, preferably no more than two

mismatches with a probe selected of a group composed of probes having a sequence GCCTGCCAGTTTCGAATGA or (SEQ ID No.1)

GTAGCCCTACTCGTAAGGA or GAGCAAAGGTATTAACCTTTACTCCC or (SEQ ID No.2)

GTTAGCCGTCCTTCTGGAG or TTATCCCCCTCTGATGGC or (SEQ ID No.5)

AGAGAAGCAAGCTCTCGTCGG or GCCACTCCTCTTTCGG or (SEQ ID No.7)

GCTAAATGCAGGGCGGATCC or CGGAAGGGCAAGGCTCTA or (SEQ ID No.9)

ACAGAAGCAAGCTCTCGTCGG, or (SEQ ID No.10)

Cancelled per (C)

26. A diagnostic test kit comprising means for detecting or identifying a bacterium suspected of being present in a sample using a method according to anyone of claims 1 to 23 or using a probe according to claim 24 or 25.

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per C